

No evidence for incipient speciation by selfing in North American *Arabidopsis lyrata*

Courtney E. Gorman¹  | Yan Li² | Marcel E. Dorken³  | Marc Stift¹ 

¹Department of Biology, University of Konstanz, Konstanz, Germany

²Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China

³Department of Biology, Trent University, Peterborough, ON, Canada

Correspondence

Courtney E. Gorman, Department of Biology, University of Konstanz, Konstanz, Germany.

Email: courtney.gorman@uni-konstanz.de

Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Number: 388824194

Abstract

Self-fertilization inherently restricts gene flow by reducing the fraction of offspring that can be produced by inter-population matings. Therefore, mating system transitions from outcrossing to selfing could result in reproductive isolation between selfing and outcrossing lineages and provide a starting point for speciation. In newly diverged lineages, for example after a transition to selfing, further reproductive isolation can be caused by a variety of prezygotic and post-zygotic mechanisms that operate before, during and after pollination. In animals, prezygotic barriers tend to evolve faster than post-zygotic ones. This is not necessarily the case for plants, for which the relative importance of post-mating, post-fertilization and early-acting post-zygotic barriers has been investigated far less. To test whether post-pollination isolation exists between populations of North American *Arabidopsis lyrata* that differ in breeding (self-incompatible versus self-compatible) and mating system (outcrossing versus selfing), we compared patterns of seed set after crosses made within populations, between populations of the same mating system and between populations with different mating systems. We found no evidence for post-pollination isolation between plants from selfing populations (self-compatible, low outcrossing rates) and outcrossing populations (self-incompatible, high outcrossing rates) via either prezygotic or early-acting post-zygotic mechanisms. Together with the results of other studies indicating the absence of reproductive barriers acting before and during pollination, we conclude that the transition to selfing in this study system has not led to the formation of reproductive barriers between selfing and outcrossing populations of North American *A. lyrata*.

KEYWORDS

Arabidopsis lyrata, mating system, post-pollination reproductive isolation, post-zygotic isolation, selfing

Konstanzer Online-Publikations-System (KOPS)
URL: <http://nbn-resolving.de/urn:nbn:de:bsz:352-2-1k4o6wuflj2oa2>

Data deposited at Dryad: <https://doi.org/10.5061/dryad.ttdz08kx6>.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Journal of Evolutionary Biology* published by John Wiley & Sons Ltd on behalf of European Society for Evolutionary Biology.

1 | INTRODUCTION

Mating system transitions from obligately outcrossing to predominantly selfing are one of the most frequent evolutionary shifts in angiosperms and have occurred repeatedly across almost all major plant lineages (Barrett, 2002). The two main benefits of selfing are thought to be transmission advantage (Fisher, 1941) and reproductive assurance in pollinator and/or mate limited situations (Darwin, 1876; Grossenbacher et al., 2017). However, there are also substantial costs associated with selfing, such as inbreeding depression (Charlesworth & Willis, 2009), as well as pollen (Holsinger et al., 1984; Nagylaki, 1976) and seed discounting (Busch & Delph, 2012; Lloyd & Schoen, 1992). Therefore, selfing should become prevalent only in situations where the advantages outweigh the fitness consequences of pollen and seed discounting, and inbreeding depression. However, while there are several situations where selfing might be favoured in the short-term, such as when colonizing a new environment (Baker, 1955; Grossenbacher et al., 2017; Stebbins, 1957), it is unclear whether selfing is advantageous over longer evolutionary timescales. It has been hypothesized that shifts from outcrossing to selfing should significantly reduce the evolutionary potential of populations due to the steep reduction in genetic diversity that accompanies selfing (Cheptou, 2019; Stebbins, 1957). However, despite being potentially disadvantageous over macroevolutionary timescales (Goldberg et al., 2010), mating system transitions and subsequent rapid reproductive isolation frequently give rise to new lineages of selfing plants.

It is estimated that approximately 15% of vascular plants are predominantly selfing and many share a relatively recent common ancestor with an outcrossing species (Goodwillie et al., 2005). These include several cases of apparent rapid speciation (Briscoe Runquist et al., 2014; Foxe et al., 2009; Guo et al., 2009). For example, the self-compatible and highly selfing *Capsella rubella* is reproductively isolated from its outcrossing and self-incompatible congener *C. grandiflora*, and this isolation has evolved within the past 20,000 years (Foxe et al., 2009). In some lineages, the transition to self-compatibility has occurred even more recently and populations of the same species are polymorphic for breeding system (self-compatibility versus self-incompatibility) and for mating system (highly outcrossing versus highly selfing; Foxe et al., 2010; Busch et al., 2011). For these plants with polymorphic breeding systems, the transition to self-compatibility and high rates of selfing might promote reproductive isolation and incipient divergence among populations.

Reproductive isolation under selfing can arise in several ways. Most directly, selfing reduces cross-pollen transfer and gene flow among populations (Brys et al., 2014; Wright et al., 2013). Selfing can also indirectly contribute to reproductive isolation (Coyne & Orr, 2004) if the evolution of selfing is accompanied by changes towards the so-called selfing syndrome, which describes the typical floral morphology and reproductive allocation that often characterize selfing plant species (Sicard & Lenhard, 2011). This syndrome likely optimizes the reproductive output of selfing plants and could

also promote reproductive isolation, both among newly evolved selfing lineages and between selfing lineages and their outcrossing ancestors (Cutter, 2019). For example, reduced pollen production and/or pollen discounting that significantly reduces the amount of pollen transferred to conspecifics due to high rates of selfing in self-compatible populations can result in reinforcement of isolating mechanisms (Rauscher, 2017). Furthermore, selfing might accelerate divergence among populations by reducing effective population size, thereby increasing the effects of genetic drift (Heller & Smith, 1978; Wright et al., 2013). Following reductions in gene flow among populations, reproductive isolation between selfing and outcrossing populations can be enforced by genetic incompatibilities (e.g. B-D-M incompatibilities between species of *Mimulus*; Bateson, 1909; Dobzhansky, 1937; Muller, 1942; Fishman & Willis, 2001). In spite of the importance of self-fertilization as a potential driver of speciation in plants, there have been relatively few studies of reproductive barriers in newly arisen lineages of selfing plants.

Reproductive barriers can operate before, during and after mating. In animal systems, such barriers are typically characterized as prezygotic (i.e. mechanisms that reduce zygote formation) or postzygotic (i.e. mechanisms that reduce the viability of hybrids and thus act after fertilization). In plants, however, it is more precise to distinguish between pre- and post-pollination mechanisms. Prepollination mechanisms are exclusively prezygotic and include differences in geography, phenology and/or pollinator attraction. However, post-pollination mechanisms can have both pre- and post-zygotic components and consist of differences in F1 seed formation and/or viability (Baack et al., 2015). Reproductive isolation in plants commonly occurs via a combination of pre- and post-pollination mechanisms (Baack et al., 2015; Coyne & Orr, 2004; Rieseberg & Willis, 2007). Prepollination mechanisms are generally expected to contribute more to total reproductive isolation after the transition to selfing (Baack et al., 2015; Widmer et al., 2009), as they act earlier in the life cycle and are often individually stronger than post-pollination barriers (Coyne & Orr, 2004; Lowry et al., 2008; Ramsey et al., 2003). However, post-pollination mechanisms have likely been underappreciated as drivers of speciation due to their relatively cryptic nature (Yost & Kay, 2009).

Reproductive barriers can be asymmetric in plants (Tiffin et al., 2001; Turelli & Moyle, 2007), and differences in mating system often play a role in such asymmetry through post-pollination mechanisms. For example, several self-compatible species can hybridize with related self-incompatible species as the female (ovule) parent, but not as the male (pollen) parent due to inhibition of pollen tube growth in the style (e.g. *Dendrobium* spp., Pinheiro et al., 2015; Lewis & Crowe, 1958). Additionally, cyto-nuclear incompatibilities (i.e. incompatibilities between organellar and nuclear genes) between the parental genomes can result in asymmetric post-zygotic reproductive isolation (Caruso et al., 2012; Tiffin et al., 2001). Furthermore, conflicts between female and male parents over resource allocation to offspring can result in asymmetric post-pollination reproductive isolation between self-compatible and self-incompatible lineages (Brandvain & Haig, 2005; Willi, 2013). However, surprisingly few

studies have attempted to quantify the relative importance of different post-pollination mechanisms of reproductive isolation, and the strength of possible asymmetries as potential drivers of speciation after the evolution of selfing (Pickup et al., 2019; Widmer et al., 2009).

Here, we use measures of the fertility of within- and between-mating system crosses of *Arabidopsis lyrata* ssp. *lyrata* to examine mechanisms of post-pollination reproductive isolation in a recently diverged selfing lineage. *Arabidopsis lyrata* has become a popular system for studying the early dynamics of the evolutionary shift to selfing, because it displays intraspecific breeding and mating system variation in its North American populations (Mable et al., 2005). Most North American populations are self-incompatible and outcrossing (multi-locus outcrossing rates $t_m > 0.8$), but in several populations all plants are self-compatible, and reproduce primarily through selfing (t_m : 0.1–0.4; Mable et al., 2005; Mable & Adam, 2007; Foxe et al., 2010). The self-incompatible and self-compatible populations are geographically interspersed and do not exhibit consistent differences in population size or habitat type (Willi & Määtänen, 2011). The transition to selfing in these populations is thought to have happened recently (<10,000 years ago), as the range now occupied by the self-incompatible and self-compatible populations was mostly covered by ice during the last glacial maximum and the selfing populations do not display a clear selfing syndrome (Carleial et al., 2017a). Furthermore, these populations likely represent multiple transitions to selfing within different population genetic backgrounds (Foxe et al., 2010; Mable et al., 2017; Table S1). Due to the multiple intraspecific origins of selfing, *A. lyrata* is ideally suited for examining the role of post-pollination mechanisms of reproductive isolation in recently diverged selfing lineages.

In this study, we examined post-pollination reproductive isolation in *A. lyrata* by quantifying patterns of seed set of crosses involving self-compatible (SC) plants from populations with high rates of selfing (hereafter: SC populations) and self-incompatible (SI) plants from populations with high rates of outcrossing (hereafter: SI populations). Specifically, we compared crosses within and between populations with the same mating system and between populations with a different mating system. The latter was done reciprocally, which allowed us to assess potential asymmetric reproductive barriers between SC and SI populations. Specifically, we compared seed number and average seed weight to assess reproductive isolation due to post-pollination mechanisms. As a baseline, we tested whether (a) populations of the same breeding and mating system were isolated from one another. Because we found some evidence for differences in seed production for within- versus between-population crosses, we examined whether (b) these differences were greater among SC than among SI populations. To test whether there is reproductive isolation between SC and SI populations we asked whether there is (c) more isolation between populations that differ in mating system than between populations of the same mating system; and (d) asymmetric isolation between SI and SC populations.

2 | METHODS

2.1 | Study system

Arabidopsis lyrata ssp. *lyrata* (L.) O'Kane & Al-Shehbaz is a small, short-lived perennial that is native to North America. It occurs in dry-mesic habitats with shallow soils, such as rock outcrops and sand dunes. Individual plants can produce several stems that terminate in racemes of numerous (>20) small white flowers. Each fruit (silique) can produce up to around 40 seeds. The ancestral condition in *A. lyrata* is self-incompatibility (multi-locus outcrossing rates $t_m > 0.8$, Table S1); however, the barrier to self-fertilization has broken down in several North American populations (Mable et al., 2005). Additionally, these newly formed self-compatible populations have evolved high rates of selfing (t_m : 0.1–0.4; Table S1) (Foxe et al., 2010).

2.2 | Crossing design

To generate the material needed to test whether SI and SC populations are reproductively isolated via post-pollination mechanisms, we first sowed field-collected seeds from 12 North American *A. lyrata* populations with known breeding (SC versus SI) and mating systems (Foxe et al., 2010; seeds were kindly provided by Barbara Mable, University of Glasgow). These included six populations with high outcrossing rates, high frequency of SI individuals, hereafter referred to as SI populations, and six populations with low outcrossing rates, high frequency of SC individuals; hereafter SC populations (Figure 1, Table S1). Plants were grown in a growth chamber set to keep 40%–60% humidity, with temperatures between 17°C and 21°C during 16 hr days and at 15°C during the night. We performed controlled crosses in 2014 (plants A, B and C, Table S2) and 2015 (plants D, E and F, Table S2) with six plants of each of the six SI and six SC populations. This crossing design produced progeny with the following cross types: within SI population (SI \times SI_{within}, $n = 141$), within SC population (SC \times SC_{within}, $n = 82$), between SI population (SI \times SI_{between}, $n = 365$), between SC population (SC \times SC_{between}, $n = 384$) and between SC and SI population reciprocally (SI \times SC, $n = 526$ or SC \times SI, $n = 272$); see electronic Table S2 for full details. A total of 1,770 crosses were made.

2.3 | Seed yield and weight

To obtain the average number of zygotes per fruit for each cross type, we first determined whether each of the 1,770 crosses produced seeds, and then counted all seeds (including seeds that appeared to have been aborted prematurely). To obtain the average seed mass per fruit, we pooled all seeds per fruit and excluded fruits that did not produce seeds. We then weighed all seeds per fruit to the nearest μg using a microbalance (Mettler Toledo XP2U).

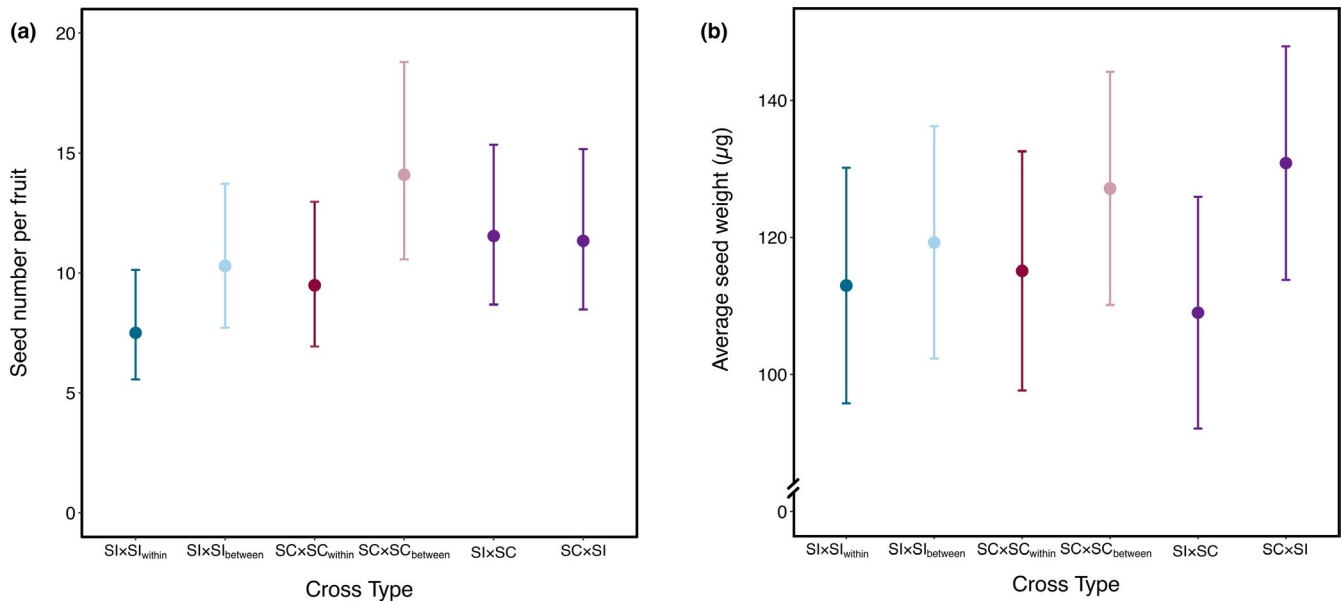


FIGURE 1 (a) Effect plot of seed number per fruit by cross type ($n = 1,770$). (b) Effect plot of average seed weight by cross type ($n = 1,667$). Predictions from the generalized linear mixed-effects models were obtained with the *ggpredict* function in the *ggeffects* R package (Lüdtke, 2018). Coloured points are predicted values, and error bars represent 95% confidence intervals

2.4 | Statistical analyses

All statistical analyses were done using R (v. 3.5.1; R Core Team, 2019). To test whether seed number per fruit differed among cross types, we used a generalized linear mixed-effects model with a Poisson distribution implemented in *lme4* (Bates et al., 2015) with *cross type* as a fixed effect, and *maternal ID* (nested within *maternal population*) and *paternal ID* (nested within *paternal population*) as random effects. To test whether the average seed weight of the crosses that produced seeds ($n = 1,667$ out of 1,770) differed among cross types, we used a Gaussian linear mixed-effects model with *cross type* as a fixed effect and the same random-effects structure described above.

To make post hoc comparisons between specific cross type combinations, we used the *glht* function in the *multcomp* package (see Table 1) (Hothorn et al., 2008) with a custom contrast matrix to test whether (C1a) SI populations are isolated from each other via post-pollination mechanisms; (C1b) SC populations are isolated from each other via post-pollination mechanisms; (C2) SC populations are more isolated from each other than SI populations; (C3) there is isolation due to mating system differences; and (C4) there is asymmetric isolation between SI and SC populations.

3 | RESULTS

Out of the 1,770 crosses, 1,667 (94%) produced seeds, and cross success was >90% in all cross types (per cent of successful crosses by cross type: SIxSI_{within}: 92.2%, SCxSC_{within}: 92.7%, SIxSI_{between}: 91.2%, SCxSC_{between}: 96.9%, SIxSC: 96.0%, SCxSI: 92.3%). Cross type had a

significant effect on both seed number and seed weight (see Table S3 for model summary statistics). With regard to the baseline comparisons, plants from different SI populations showed no isolation from each other via post-pollination mechanisms, because progeny resulting from crosses between SI populations did not have a lower seed set or seed weight than progeny from crosses within SI populations. The same was true for SC populations. In fact, crosses between populations yielded a higher seed number per fruit (SIxSI: 20.5% increase; SCxSC: 39.2% increase) and higher seed weight per seed (SIxSI: 4.8% increase; SCxSC: 11.4% increase) than crosses within populations (significant effects of C1a and C1b Table 1, Figure 1). This beneficial effect of inter-population crosses was 3–4× larger for crosses between SC populations than for crosses between SI populations, although the difference in magnitude was not significant (no significant effect of C2 Table 1, Figure 1). In other words, there was no evidence for more isolation between SC populations than between SI populations.

On average, crosses between populations that differed in mating system (SIxSC and SCxSI cross types) yielded similar seed numbers and slightly (1.6%) smaller seeds compared with crosses between populations of the same mating system (C3 in Table 1). In other words, populations were not isolated more from each other if they differed in mating system in terms of seed number and only slightly so in terms of seed weight.

Although crosses tended to yield more seeds and larger seeds when the maternal cross-partner was SC (SIxSC: 13.9 versus SCxSI: 15.0 seeds per fruit; SIxSC: 110.5 versus SCxSI: 131.6 µg per seed), cross-direction did not have a significant effect on seed number and seed weight (no significant effects of C4, Table 1, Figure 1). In other words, there was no significant asymmetric isolation between SI and SC populations.

TABLE 1 Post hoc hypothesis testing for seed traits using a contrast matrix (*g/ht* function in the multcomp package; Hothorn et al., 2008)

Post hoc comparison	Seed number		Seed weight		Contrast definitions							
	Estimate ± SE	<i>z</i>	<i>Pr</i> (> <i>z</i>)	Estimate ± SE	<i>z</i>	<i>Pr</i> (> <i>z</i>)	SI × SI _{within}	SI × SI _{between}	SC × SC _{within}	SC × SC _{between}	SI × SC	SC × SI
Baseline comparisons of within-versus between-population crosses												
C1a: Isolation between SI populations? ^a	0.32 ± 0.07	4.85	<0.001	6.28 ± 2.09	3.00	0.012	-1	1	0	0	0	0
C1b: Isolation between SC populations? ^a	0.40 ± 0.08	5.08	<0.001	12.03 ± 2.56	4.71	<0.001	0	0	-1	1	0	0
C2: Difference in magnitude of effect C1a and C1b? ^b	-0.04 ± 0.05	-0.79	0.898	-2.87 ± 1.65	-1.74	0.302	-0.5	0.5	0.5	-0.5	0	0
Hypothesis testing												
C3: Isolation due to mating system differences? ^c	-0.05 ± 0.03	-1.54	0.424	-3.27 ± 1.11	-2.95	0.015	0	-0.5	0	-0.5	0.5	0.5
C4: Asymmetric isolation between mating systems? ^d	0.02 ± 0.21	0.09	1.000	-21.81 ± 12.25	-1.78	0.282	0	0	0	0	1	-1

Note: Significant effects are highlighted in bold.

Abbreviations: SC, self-compatible; SE, standard error; SI, self-incompatible; *z*, Wald statistic.

^aA negative parameter estimate would indicate reproductive isolation (i.e. outbreeding depression) between populations. A positive estimate would indicate the opposite (i.e. a benefit of inter-population outbreeding).

^bA negative estimate would indicate that the magnitude of C1a is smaller than the magnitude of C1b. A positive estimate would indicate the opposite (that the magnitude of C1a is larger than the magnitude of C1b).

^cA negative estimate would indicate reproductive isolation between breeding systems. A positive estimate would indicate the opposite (i.e. a benefit of inter-population outbreeding).

^dA significant estimate would indicate asymmetry in reproductive isolation between breeding systems. A positive estimate would indicate that crosses with SI mothers have higher trait values than crosses with SC mothers (i.e. SI × SC > SC × SI). A negative estimate would indicate the opposite, that is that crosses with SI mothers have lower trait values than crosses with SC mothers (i.e. SI × SC < SC × SI).

4 | DISCUSSION

Our main finding is that SI and SC populations of North American *A. lyrata* ssp. *lyrata* are not reproductively isolated from each other via post-pollination mechanisms. To the contrary, both for the set of SI and the set of SC populations, we found that seed number and seed weight were higher when plants from different populations were crossed. The benefits of crossing between-populations were of similar magnitude between the breeding systems. Thus, our results effectively show the opposite of what we would expect if the evolution of selfing had driven the evolution of reproductive isolation among the studied SC and SI populations. Moreover, comparing the reciprocal SI \times SC and SC \times SI cross types provided no evidence of asymmetric pre- or post-zygotic post-pollination barriers between SI and SC populations, as cross direction did not significantly influence seed number or seed weight.

4.1 | No evidence for reproductive isolation of newly evolved selfing populations

The lack of reproductive barriers between SI and SC populations in terms of seed number and seed weight and the lack of evidence for increased reproductive isolation between SC populations clearly shows that the transition to selfing has not led to the formation of reproductive barriers. By contrast, two subspecies of *Clarkia xantiana* that differ in mating system are almost completely reproductively isolated despite being recently diverged (ca. 65,000 years ago) (Briscoe Runquist et al., 2014). Similarly, an even more recent divergence (ca. 20,000–50,000 years ago), the selfing *Capsella rubella* is considered a separate species from *Capsella grandiflora* based on contrasting floral morphologies and near complete reproductive isolation (Fuxe et al., 2009; Guo et al., 2009; Sicard & Lenhard, 2011). *Arabidopsis lyrata* differs from these systems in that (a) plants within SC populations of *A. lyrata* do not have features associated with the selfing syndrome (Carleial et al., 2017a) and (b) SI and SC populations are not reproductively isolated via pre-pollination mechanisms (Gorman, Bond, et al., 2020). Since the transition to selfing in SC populations of *A. lyrata* likely occurred in the last 10,000 years (Fuxe et al., 2010), it is possible that there has not been enough time for the evolution of reproductive barriers between these populations, although there is some evidence that the SC populations have diverged from the SI populations in their life history (lifespan; Gorman, Steinecke, et al., 2020). Therefore, reproductive barriers, especially if they are influenced by lifespan, could become stronger as SC populations continue to diverge from SI populations.

4.2 | No asymmetric reproductive isolation between mating systems

Our finding that reciprocal crosses between SI and SC populations do not yield differences in seed number and seed weight show that

there are no asymmetric post-pollination barriers between SI and SC *A. lyrata* populations. This contrasts with a body of previous theoretical and empirical work suggesting that asymmetric post-pollination barriers to reproduction are common among plants with contrasting mating systems (Brandvain & Haig, 2005; Briscoe Runquist et al., 2014; Lewis & Crowe, 1958; Pinheiro et al., 2015; Willi, 2013). For example, two subspecies of *Clarkia xantiana* that differ in mating system are nearly completely reproductively isolated via several pre- and post-mating barriers and have strong asymmetry in seed production, as SI \times SC crosses produce substantially more viable seeds than SC \times SI crosses (Briscoe Runquist et al., 2014). *Arabidopsis lyrata* thus differs from this general pattern (but see Willi, 2013), and it also did not display any sign of asymmetric reproductive isolation through differences in pollinator attraction or phenology (Gorman, Bond, et al., 2020). Since biased cross-compatibilities tend to arise in the presence of other isolating barriers and/or post-speciation (Pinheiro et al., 2015), it is possible that the transition to self-compatibility in SC populations of *A. lyrata* is too recent for these kinds of obvious asymmetries to develop. Despite the lack of divergence for other traits, we recently found that SC populations have a reduced lifespan (Gorman, Steinecke, et al., 2020). Over longer evolutionary timescales such asymmetries could become stronger and contribute substantially to the reproductive isolation between SI and SC populations. However, our finding that cross-direction for SI \times SC and SC \times SI crosses had no effect on seed number or weight underscores that there are currently no strong asymmetrically acting post-pollination barriers between SI and SC *A. lyrata* populations.

4.3 | Potential consequences of secondary contact

We found that seeds produced by between-mating system crosses (i.e. SI \times SC and SC \times SI) had similar seed number and seed weight to plants produced by the between-population within mating system crosses (i.e. SI \times SI_{between} and SC \times SC_{between}). Previous work on pre-pollination mechanisms of reproductive isolation in *A. lyrata* found that plants produced by between-mating system crosses had similar phenological traits to plants produced by within-mating system crosses and that the two mating system types substantially overlapped in phenology and lacked pollinator specificity (Gorman, Bond, et al., 2020). Taken together, this indicates that in a scenario of secondary contact (i.e. SI and SC populations coming into contact with one another), SI and SC populations would likely merge. Secondary contact between SC and SI populations should therefore yield ample opportunity for cross-pollination. The absence of post-pollination barriers to interbreeding between plants from SC and SI populations found here indicates that it is likely that in a scenario of secondary contact *A. lyrata* would initially maintain a mixed-breeding system containing a mixture of SI and SC plants (Goodwillie et al., 2005). Self-fertilization is associated with relatively low levels of inbreeding depression in *A. lyrata* (Carleial et al., 2017b; Li et al., 2019). Therefore, admixed

populations with a mixed-breeding system could theoretically also maintain a stable mixed mating system. The outcome will ultimately depend on differences in relative performance between SI and SC plants in interplay with ecological factors such as mate and pollinator limitation.

5 | CONCLUSIONS

Our experiment showed that SI and SC populations of *A. lyrata* are not strongly reproductively isolated via pre- or post-zygotic post-pollination mechanisms that affect seed set. To the contrary, crossing between populations (irrespective of their mating system) had a considerable benefit over crossing within population. Since we only examined seed number and seed weight of F1 progeny, our findings cannot rule out genetic incompatibilities that only become detectable in later generations (Dobzhansky, 1937; Fishman & Willis, 2001; Muller, 1942). However, a follow-up experiment showed that seeds formed by F1 plants in a common garden were as viable as seeds formed by the parental plants (C. E. Gorman, M. E. Dorken, and M. Stift, unpublished data). Thus, combined with previous findings that SI and SC *A. lyrata* populations are not strongly reproductively isolated by pre-pollination mechanisms (Gorman, Bond, et al. 2020), we conclude that the selfing populations in North American *A. lyrata* show little to no detectable signs of incipient speciation.

ACKNOWLEDGEMENTS

Barbara Mable provided source seed material. Ekaterina Mamonova and Sina Konitzer-Glöckner helped with seed production. Elena Werner and Karoline Jetter helped with counting and weighing seeds. This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—project 388824194 to M.S.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

M.S. conceived the project and designed the experiment. C.E.G. and Y.L. collected the data. C.E.G. and M.S. analysed the data with input from M.D. C.E.G., M.D. and M.S. wrote the paper with input from all authors. All authors have approved the final version of the manuscript and agree to be held accountable for the content therein.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13901>.

DATA AVAILABILITY STATEMENT

The data associated with this manuscript are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.ttdz08kx6>.

ORCID

Courtney E. Gorman  <https://orcid.org/0000-0001-7310-8803>

Marcel E. Dorken  <https://orcid.org/0000-0001-7400-5136>

Marc Stift  <https://orcid.org/0000-0001-7801-9498>

REFERENCES

- Baack, E., Melo, M. C., Rieseberg, L. H., & Ortiz-Barrientos, D. (2015). The origins of reproductive isolation in plants. *New Phytologist*, 207(4), 968–984. <https://doi.org/10.1111/nph.13424>
- Baker, H. G. (1955). Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, 9(3), 347–357. <https://doi.org/10.2307/2405656>
- Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews Genetics*, 3(4), 274–284. <https://doi.org/10.1038/nrg776>
- Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://www.jstatsoft.org/v067/i01>
- Bateson, W. (1909). *Heredity and variation in modern lights*. Cambridge University Press.
- Brandvain, Y., & Haig, D. (2005). Divergent mating systems and parental conflict as a barrier to hybridization in flowering plants. *The American Naturalist*, 166(3), 330–338. <https://doi.org/10.1086/432036>
- Briscoe Runquist, R. D., Chu, E., Iverson, J. L., Kopp, J. C., & Moeller, D. A. (2014). Rapid evolution of reproductive isolation between incipient outcrossing and selfing *Clarkia* species. *Evolution*, 68, 2885–2900.
- Brys, R., Vanden Broeck, A., Mergeay, J., & Jacquemyn, H. (2014). The contribution of mating system variation to reproductive isolation in two closely related *Centaureum* species (Gentianaceae) with a generalized flower morphology. *Evolution*, 68, 1281–1293.
- Busch, J. W., & Delph, L. F. (2012). The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Annals of Botany*, 109(3), 553–562. <https://doi.org/10.1093/aob/mcr219>
- Busch, J. W., Joly, S., & Schoen, D. J. (2011). Demographic signatures accompanying the evolution of selfing in *Leavenworthia alabamica*. *Molecular Biology and Evolution*, 28(5), 1717–1729. <https://doi.org/10.1093/molbev/msq352>
- Carleial, S., van Kleunen, M., & Stift, M. (2017a). Small reductions in corolla size and pollen: Ovule ratio, but no changes in flower shape in selfing populations of the North American *Arabidopsis lyrata*. *Oecologia*, 183, 401–413.
- Carleial, S., van Kleunen, M., & Stift, M. (2017b). Relatively weak inbreeding depression in selfing but also in outcrossing populations of North American *Arabidopsis lyrata*. *Journal of Evolution Biology*, 30, 1994–2004.
- Caruso, C. M., Case, A. L., & Bailey, M. F. (2012). The evolutionary ecology of cytonuclear interactions in angiosperms. *Trends in Plant Science*, 17(11), 638–643. <https://doi.org/10.1016/j.tplan.2012.06.006>
- Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, 10(11), 783–796. <https://doi.org/10.1038/nrg2664>
- Cheptou, P.-O. (2019). Does the evolution of self-fertilization rescue populations or increase the risk of extinction? *Annals of Botany*, 123(2), 337–345. <https://doi.org/10.1093/aob/mcy144>
- Coyne, J., & Orr, H. (2004). *Speciation*. Sinauer Associates.
- Cutter, A. D. (2019). Reproductive transitions in plants and animals: selfing syndrome, sexual selection and speciation. *New Phytologist*, 224(3), 1080–1094. <https://doi.org/10.1111/nph.16075>
- Darwin, C. (1876). *The effects of cross and self fertilisation in the vegetable kingdom / by Charles Darwin*. John Murray.
- Dobzhansky, T. (1937). *Genetics and the origin of species*. Columbia University Press.

- Fisher, R. A. (1941). Average excess and average effect of a gene substitution. *Annals of Eugenics*, 11(1), 53–63. <https://doi.org/10.1111/j.1469-1809.1941.tb02272.x>
- Fishman, L., & Willis, J. H. (2001). Evidence for dobzhansky-muller incompatibilities contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. *Evolution*, 55(10), 1932–1942. <https://doi.org/10.1111/j.0014-3820.2001.tb01311.x>
- Foxe, J. P., Slotte, T., Stahl, E. A., Neuffer, B., Hurka, H., & Wright, S. I. (2009). Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences*, 106(13), 5241–5245. <https://doi.org/10.1073/pnas.0807679106>
- Foxe, J. P., Stift, M., Tedder, A., Haudry, A., Wright, S. I., & Mable, B. K. (2010). Reconstructing origins of loss of self-incompatibility and selfing in North American *Arabidopsis lyrata*: A population genetic context. *Evolution*, 64(12), 3495–3510. <https://doi.org/10.1111/j.1558-5646.2010.01094.x>
- Goldberg, E. E., Kohn, J. R., Lande, R., Robertson, K. A., Smith, S. A., & Igić, B. (2010). Species selection maintains self-incompatibility. *Science*, 330(6003), 493–495. <https://doi.org/10.1126/science.1194513>
- Goodwillie, C., Kalisz, S., & Eckert, C. G. (2005). The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 47–79. <https://doi.org/10.1146/annurev.ecolsys.36.091704.175539>
- Gorman, C. E., Bond, L., van Kleunen, M., Dorken, M. E., & Stift, M. (2020a). Limited phenological and pollinator-mediated isolation among selfing and outcrossing *Arabidopsis lyrata* populations. *Proceedings of the Royal Society B Biological Sciences*, 287, 20202323.
- Gorman, C. E., Steinecke, C., van Kleunen, M., Dorken, M. E., & Stift, M. (2020b). A shift towards the annual habit in selfing *Arabidopsis lyrata*. *Biology Letters*, 16, 20200402.
- Grossenbacher, D. L., Brandvain, Y., Auld, J. R., Burd, M., Cheptou, P., Conner, J. K., Grant, A. G., Hovick, S. M., Pannell, J. R., Pauw, A., Petanidou, T., Randle, A. M., Rubio de Casas, R., Vamosi, J., Winn, A., Igić, B., Busch, J. W., Kalisz, S., & Goldberg, E. E. (2017). Self-compatibility is over-represented on islands. *New Phytologist*, 215(1), 469–478. <https://doi.org/10.1111/nph.14534>
- Guo, Y. L., Bechsgaard, J. S., Slotte, T., Neuffer, B., Lascoux, M., Weigel, D., & Schierup, M. (2009). Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 5246–5251.
- Heller, R., & Smith, J. M. (1978). Does Muller's ratchet work with selfing? *Genetical Research*, 32, 289–293. <https://doi.org/10.1017/S0016672300018784>
- Holsinger, K. E., Feldman, M. W., & Christiansen, F. B. (1984). The evolution of self-fertilization in plants: A population genetic model. *The American Naturalist*, 124(3), 446–453.
- Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 3, 346–363. <https://doi.org/10.1002/bimj.200810425>
- Lewis, D., & Crowe, L. K. (1958). Unilateral interspecific incompatibility in flowering plants. *Heredity*, 12(2), 233–256. <https://doi.org/10.1038/hdy.1958.26>
- Li, Y., van Kleunen, M., & Stift, M. (2019). Sibling competition does not magnify inbreeding depression in North American *Arabidopsis lyrata*. *Heredity*, 123, 723–732. <https://doi.org/10.1038/s41437-019-0268-1>
- Lloyd, D. G., & Schoen, D. J. (1992). Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences*, 153(3, Part 1), 358–369. <https://doi.org/10.1086/297040>
- Lowry, D. B., Modliszewski, J. L., Wright, K. M., Wu, C. A., & Willis, J. H. (2008). The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 1506, 3009–3021. <https://doi.org/10.1098/rstb.2008.0064>
- Lüdecke, D. (2018). ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software*, 3, 772. <https://doi.org/10.21105/joss.00772>
- Mable, B. K., & Adam, A. (2007). Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. *Molecular Ecology*, 16, 3565–3580.
- Mable, B. K., Hagmann, J., Kim, S.-T., Adam, A., Kilbride, E., Weigel, D., & Stift, M. (2017). What causes mating system shifts in plants? *Arabidopsis lyrata* as a case study. *Heredity*, 118, 52–63.
- Mable, B. K., Robertson, A. V., Dart, S., Berardo, C. D., & Witham, L. (2005). Breakdown of self-incompatibility in the perennial *Arabidopsis lyrata* (Brassicaceae) and its genetic consequences. *Evolution*, 59, 1437–1448. <https://doi.org/10.1111/j.0014-3820.2005.tb01794.x>
- Muller, H. (1942). Isolating mechanisms, evolution, and temperature. *Biology Symposium*, 6, 71–125.
- Nagylaki, T. (1976). A model for the evolution of self-fertilization and vegetative reproduction. *Journal of Theoretical Biology*, 58(1), 55–58. [https://doi.org/10.1016/0022-5193\(76\)90138-7](https://doi.org/10.1016/0022-5193(76)90138-7)
- Pickup, M., Brandvain, Y., Fraïsse, C., Yakimowski, S., Barton, N. H., Dixit, T., Lexer, C., Cereghetti, E., & Field, D. L. (2019). Mating system variation in hybrid zones: facilitation, barriers and asymmetries to gene flow. *New Phytologist*, 224(3), 1035–1047. <https://doi.org/10.1111/nph.16180>
- Pinheiro, F., Cafasso, D., Cozzolino, S., & Scopece, G. (2015). Transitions between self-compatibility and self-incompatibility and the evolution of reproductive isolation in the large and diverse tropical genus *Dendrobium* (Orchidaceae). *Annals of Botany*, 116, 457–467.
- R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Ramsey, J., Bradshaw, H. D., & Schemske, D. W. (2003). Components of reproductive isolation between the monkeyflowers *mimulus lewisii* and *m. cardinalis* (phrymaceae). *Evolution*, 57(7), 1520–1534. <https://doi.org/10.1111/j.0014-3820.2003.tb00360.x>
- Rausher, M. D. (2017). Selfing, local mate competition, and reinforcement. *The American Naturalist*, 189(2), 87–104. <https://doi.org/10.1086/690009>
- Rieseberg, L. H., & Willis, J. H. (2007). Plant speciation. *Science*, 317(5840), 910–914. <https://doi.org/10.1126/science.1137729>
- Sicard, A., & Lenhard, M. (2011). The selfing syndrome: A model for studying the genetic and evolutionary basis of morphological adaptation in plants. *Annals of Botany*, 107(9), 1433–1443. <https://doi.org/10.1093/aob/mcr023>
- Stebbins, G. L. (1957). Self fertilization and population variability in the higher plants. *The American Naturalist*, 91(861), 337–354. <https://doi.org/10.1086/281999>
- Tiffin, P., Olson, M. S., & Moyle, L. C. (2001). Asymmetrical crossing barriers in angiosperms. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1469), 861–867. <https://doi.org/10.1098/rspb.2000.1578>
- Turelli, M., & Moyle, L. C. (2007). Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. *Genetics*, 176(2), 1059–1088. <https://doi.org/10.1534/genetics.106.065979>
- Widmer, A., Lexer, C., & Cozzolino, S. (2009). Evolution of reproductive isolation in plants. *Heredity*, 102(1), 31–38. <https://doi.org/10.1038/hdy.2008.69>
- Willi, Y. (2013). The battle of the sexes over seed size: support for both kinship genomic imprinting and interlocus contest evolution. *The American Naturalist*, 181(6), 787–798. <https://doi.org/10.1086/670196>
- Willi, Y., & Määttä, K. (2011). The relative importance of factors determining genetic drift: Mating system, spatial genetic structure, habitat and census size in *Arabidopsis lyrata*. *New Phytologist*, 189, 1200–1209.
- Wright, S. I., Kalisz, S., & Slotte, T. (2013). Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society B: Biological Sciences*, 1506, 3009–3021. <https://doi.org/10.1098/rstb.2008.0064>

Biological Sciences, 280(1760), 20130133. <https://doi.org/10.1098/rspb.2013.0133>

Yost, J. M., & Kay, K. M. (2009). The evolution of postpollination reproductive isolation in *Costus*. *Sexual Plant Reproduction*, 4, 247–255. <https://doi.org/10.1007/s00497-009-0113-4>

How to cite this article: Gorman, C. E., Li, Y., Dorken, M. E., & Stift, M. (2021). No evidence for incipient speciation by selfing in North American *Arabidopsis lyrata*. *Journal of Evolutionary Biology*, 34, 1397–1405. <https://doi.org/10.1111/jeb.13901>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.